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Review article

The tubercular badger and the uncertain curve:- The need for a multiple stressor approach in environmental radiation protection

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ABSTRACT

This article presents the results of a workshop held in Stirling, Scotland in June 2018, called to examine critically the effects of low-dose ionising radiation on the ecosphere. The meeting brought together participants from the fields of low- and high-dose radiobiology and those working in radioecology to discuss the effects that low doses of radiation have on non-human biota. In particular, the shape of the low-dose response relationship and the extent to which the effects of low-dose and chronic exposure may be predicted from high dose rate exposures were discussed. It was concluded that high dose effects were not predictive of low dose effects. It followed that the tools presently available were deemed insufficient to reliably predict risk of low dose exposures in ecosystems. The workshop participants agreed on three major recommendations for a path forward. First, as treating radiation as a single or unique stressor was considered insufficient, the development of a multidisciplinary approach is suggested to address key concerns about multiple stressors in the ecosphere. Second, agreed definitions are needed to deal with the multiplicity of factors determining outcome to low dose exposures as a term can have different meanings in different disciplines. Third, appropriate tools need to be developed to deal with the different time, space and organisation level scales. These recommendations permit a more accurate picture of prospective risks.

Abbreviations: CEZ, Chernobyl exclusion zone; TLD, thermal luminescence dosimeter

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1. Introduction

The natural environment is the constant recipient of a wide range of anthropogenic biological, chemical and physical insults, including radionuclides, heavy metals and organic pollutants that impact directly or indirectly on the flora and fauna (e.g. Batlle et al., 2016; Farajnejad et al., 2017). This workshop followed on from others organised by the International Union of Radioecology, which aimed to bring radiobiologists and radioecologists together to discuss issues of common interest. The emphasis of this workshop was placed on the question of the shape of the low doses of radiation. The particular emphasis of this workshop was the question of the shape of the low dose biological response relationship and to what extent low dose and chronic exposure effects at the level of the organism, population and ecosystem could be predicted from high dose acute exposures or models developed from theoretical or laboratory-based information. The title of this paper reflects the uncertainty surrounding the nature of the low dose response and the variation in response of organisms and individuals within populations. The "Tubercular badger" is a reference to the discussion which took place about reasons for the variation in response which are discussed but include other stressors, pathogens and parasites and impacts on defence systems such as the immune response.

The attention of workshop participants was focused on discussing the following issues:

1.1. Key questions

Basic radiobiological issues

1. Can we extrapolate endpoints and risk from high to low dose/dose rate or are there different mechanisms involved?
2. If there are different mechanisms, can we identify thresholds where mechanisms shift and what the consequences are of such mechanistic shifts?
3. Can we identify integrated mechanistic approaches which could guide understanding of radiation effects at low doses e.g. can we move from DNA/mutation dominated ideas in radiation protection to include involvement of other processes?
   From radiation effects on the organism to effects on the whole ecosystem
4. Can we identify robust field-based bioassays of effect?
5. What lessons can we learn from Chernobyl and Fukushima regarding extrapolations from high to low dose and dose rate?
6. What are the key knowledge gaps in developing an integrated approach to low dose risk assessment and management?
7. Can we identify population and ecosystem level biomarkers or must a "biomarker" always be measured in an organism?
8. How relevant are chemical and pathological stressors in modulating radiation effects?
   Modelling approaches and novel system level effects relevant in environmental radiation protection
9. Do any novel or hitherto unconsidered mechanisms impact on the spectrum of susceptible species in an ecosystem such as system level emergent mechanisms?
10. If we move to an ecosystem approach how do we deal with dose rate, route of exposure and duration of exposure in the environment, and how can we include concepts of ecosystem resilience, rescue effects and warning/signalling?
11. How relevant are adaptive responses and adaptation in populations?
12. Evidence suggests that in the field, organisms are more negatively impacted by radiation than in the laboratory; how do we factor this into risk modelling?

2. The discussion on these questions are summarised in the following sections

Basic radiobiological issues

2.1. Can we extrapolate endpoints and risk from high to low dose/dose rate or are there different mechanisms involved?

The workshop participants acknowledged that there are several different mechanisms that dominate at different doses and dose rates, which are associated with different endpoints. The challenge is in deciding which are relevant endpoints, doses and radiation quality. At low dose (< 100 mGy), some cells can exhibit hyper-radiosensitivity in clonogenic survival assays that appears to reflect a lack of fully functional DNA repair, whereas $> \sim 300$ mGy there is increased resistance that has been interpreted as an adaptive response (Wykes et al., 2006), which is a clear example of non-linearity in dose response. In addition, Rodrigues-Moreira et al. (2017) reported persistent oxidative stress in long term cultured hematopoietic stem cells exposed to low-dose radiation that was under control of the Keap1/Nrf2 antioxidant pathway. This seemed not to trigger the ATM-dependent DNA damage response typical of DNA double strand breaks seen at higher doses. The suggestion was that a relevant response endpoint may be radiation-induced senescence with continued intermittent ROS production. Since the Nrf2 pathway drives metabolic reprogramming towards use of the pentose phosphate pathway, is heavily influenced by MAP kinase signalling, and promotes anti-inflammatory immune responses. It is unlikely that all cells respond similarly, but the concept is important. It is reasonable to suggest that as radiation dose approaches levels more relevant to accidental environmental exposure, disturbances in redox regulation become more important than the DNA damage response seen after higher doses, and that environmental and metabolic influences will be more significant. As a result, the contribution of non-targeted effects and bystander mechanisms associated with acute and delayed effects may become more dominant (with triggering doses in the range 2–5 mGy (Liu et al., 2006; Schettino et al., 2006) and saturation levels around 500 mGy (Seymour and Mothersill, 2000; Prise et al., 2002, 2003; Liu et al., 2006). The generation of mutations from misrepaired/unrepaired DNA or epigenetic/paramutational mechanisms may be expected to produce the increased variation on which population adaptation is based. However, the rapidity with which phenotypic change of populations has been noted is too fast in many cases for the classical evolutionary synthetic model to explain.

The relationship of the sensitivity curves for laboratory experiments and field data on a range of species (Garnier-Laplace et al., 2013) suggests that in the wild species are much more sensitive suggesting that there are sensitising factors, such as predation, competition, food limitation, and other forms of environmental stress, some of which might be nutritional or due to multistressors like metals. Interplay between these in terms of outcomes and parallel or convergent mechanisms and targets means that it is very difficult if not impossible to extrapolate risk from high doses to the kinds of doses and dose rates to which species are exposed in the wild. Similar confounders are the differential exposure of internal and external irradiation where the relative biological effectiveness of a given dose may be different depending on how and where it is delivered. Consequently, extrapolation to specific endpoints and dose response relationships may be different for different species.

2.2. If there are different mechanisms, can we identify breakpoints where mechanisms shift and what the consequences are of the mechanistic shifts?

There is considerable evidence for breakpoints or thresholds in dose effect curves suggesting underlying mechanisms where transitions occur. New mechanisms begin to dominate as the dose increases and previously dominant mechanisms decline. Evidence from Mothersill/

Seymour's laboratory using normal urothelial explants identified a clear breakpoint at 0.5 Gy where the radiosensitivity became less in terms of incremental death with increasing dose. In a cohort of paediatric patients, the effect was pronounced (Mothersill et al., 1997). This suggests a mechanistic shift towards "saving" cells from death at higher doses, which may be important for population survival. For non-targeted effects (bystander effect and genomic instability), there are clearly thresholds at 2 mGy (turn on of signalling mechanism) and at 0.5 Gy – (saturation of signalling mechanism). These effects have been seen in several systems but not in vivo (Liu et al., 2006; Schettino et al., 2005, 2006) although they have been recorded *ex vivo* in human explants (Harney et al., 1995; Mothersill et al., 2001). hyper- radiosensitivity and induced radioresistance transition has been documented in vivo and in vitro as a low-dose effect where there is hypersensitivity to low doses. Full dose response data are available for cells and explants in culture (Joiner et al., 1993, 1996; Mothersill et al., 1999, 2001; Turesson et al., 2010; Fernandez-Palomo et al., 2016). Here the transition is around 50–100 mGy in the systems which have been investigated. Similarly, a non-linear dose-effect relationship between the frequency of aberrant cells and dose with a dose-independent plateau in the range of 50–500 mGy was observed in the root meristem of barley seedlings (Geras'kin et al., 2007). In natural populations such detailed dose-effect curves have not been generated but there is ample evidence from the work of Møller and Mousseau of greater than expected sensitivity to low-dose exposures in field conditions (Møller et al., 2011, 2012). Such transitions have also been observed in stem cells, affecting quiescent stem cell activity (Jeggo, personal communication).

The importance of these transition points is that they suggest non-linear responses and the induction of different coping mechanisms at different doses. Presumably, they vary with species, time post-exposure, chronic exposures, life cycle stage and in in vivo situations they will be highly dependent on other stressors present in the environment. There is some evidence that heavy metals strongly affect expression of non-targeted effects, and there are many instances where chronic exposures result in altered patterns of expression of non-targeted effects (Mothersill et al., 2007; Salbu et al., 2008). The practical or theoretical importance is that they suggest unknown mechanisms which shift the pattern of dose response to benefit the survival of functional aspects of tissues in order to avoid collapse. It is not possible to say whether these systems operate at higher levels such as communities or ecosystems, but it is likely that they do.

In the *ex vivo* work done on tissues and cells, there was clear inter-individual variation and also a difference between smoker/non-smoker responses. Males and females have different quantitative expression and paediatric samples were the most radiosensitive in the low dose region (Harney et al., 1995; Mothersill et al., 1999). Different cell lines showed different transition points. Even at the population and ecosystem levels, it is highly likely (Geras'kin, 2016) that non-linearity rather than linearity dominates the dose-response curve. The direction of displacement in non-linear curves is likely to be highly variable and dominated by what else is going on in the system. Therefore, maybe we need to be less concerned about "reducing uncertainty" and more concerned about determining the range of responses under different circumstances, i.e. focus not on "uncertainty" but rather on "variability". Measuring range variation might be a robust population-level metric. A further issue affecting the shape of the dose-effect curve is going to be the level of resilience or redundancy in the system – i.e. the flexibility of the system to absorb change. This leads to the possibility that the homeostatic zone could become an important metric as well. Further modulators of the low-dose response might be the history of stress – adaptive responses developed against other stressors might be readily inducible in pre-stressed systems but not in pristine systems. This type of "cross resistance" is well known in bacteria where resistance to desiccation or high temperature can also provide resistance to radiation (Diaz and Schulze-Makuch, 2006). Multiple stressor impacts clearly dominate at low dose response and will be major contributors to variation.

2.3. Can we identify integrated mechanistic approaches which could guide understanding of radiation effects at low doses e.g. can we move from DNA/mutation dominated ideas in radiation protection to include involvement of other processes?

To consider integrated mechanisms affecting radiosensitivity, insight can be gained from discussing current analysis of the response of humans following radiation exposure. Doses encountered by the normal tissue can range from μGy to mGys, which is generally larger than considered here, but nonetheless insight can be gained. It is well reported that 5–10% of radiotherapy patients have a heightened response relative to the average patient, regardless of tumour site or hospital where radiation exposure is carried out. Such patients can be classified as radiosensitive (for that tissue). Most of the processes/genes affecting the response to ionizing radiation-induced DNA damage are known and mutational changes are found in patients displaying the most marked overresponse (grade 5). But they represent a tiny fraction of the over-responding patients (although they represent a significant fraction of those showing the most marked response). Nonetheless, the majority of overresponding patients do not have mutations, single nucleotide polymorphisms or heterozygosity in the known damage response genes. So, something else confers radiosensitivity. Assays have been established that are reported to predict radiosensitivity, although rigorous consolidation of these findings is required. However, an endogenously elevated stress response is one feature that could explain the findings of all assays. To gain insight, scientists involved in MELODI (multidisciplinary European low dose initiative; Salomaa et al., 2017) are seeking an integrated approach to establish a prospective cohort of patients and record information that includes the magnitude of the response, the tumour site, the endogenous level of stress and ability to induce it post radiation (one assay involves 8-Oxo-dG levels in the plasma), the immune response (including innate immunity), life style factors, epigenetic parameters, inflammation. All these factors could impact upon radiation sensitivity. This will provide insight into factors influencing radiosensitivity in the human population. Nonetheless, this could be useful to assess the impact of factors such as immunity, inflammation and stress on the tissue response to ionizing radiation. While concerns in radioecology centre on responses at the level of populations, communities and ecosystems, it is still important in trying to develop ecosystem approaches in radiation protection that the fundamental mechanisms operating at the level of individual organisms within those higher level groups are understood. The challenge is to understand how the basic mechanisms play out at higher organisational levels. Human data, while it may seem irrelevant for ecosystem level protection, do point to regulatory systems which are modified by low dose exposure and allow the identification of potentially useful endpoints and being very well studied and robust, provide a source of radiobiological information not available for other species.

If mutation is a significant endpoint at the relevant low doses and dose rate, then an understanding of how factors such as oxidative stress, replicative stress, the immune response affects the fidelity of repair of ionizing radiation induced lesions is required e.g. associated base damage from oxidative stress could make ionizing radiation induced DNA double strand breaks more complex and hence diminish the accuracy of repair. The capacity to double strand breaks repair is high but the fidelity of repair and how it is influenced by other factors (other stresses or other DNA damaging agents) is poorly understood. To gain some insight into this, next generation sequencing is a useful tool that could be applied.

It is unlikely that mutagenic changes (direct or indirect) can contribute to widespread population phenotype changes over a relatively small number of generations, simply because the number of mutagenic hits (the vast majority of which are unlikely to be adaptive) will be insufficient. This leaves us with the possibility that there might be sufficient pre-existing pre-adapted sub-populations, which are selected following environmental change. Selective pressures may either be the

impact of radiation itself on fitness, or the impact of radiation on other organisms in the ecosystem with the concomitant perturbation; i.e. indirect effects. Epigenetic variation, either pre-existing or caused by radiation exposure (epi- or paramutation) has been associated with preadaptive "phenotypic plasticity' and may underlie one model in which phenotypic change is very fast and happens in a large number of organisms in the population, providing a "buffer' to support the generation of further genetic variance and rapid selection under changing environmental conditions than might be otherwise predicted (Nishikawa and Kinjo, 2014, 2018) although this is hotly debated (Geoghegan and Spencer, 2013; Gjuvsland et al., 2016; Charlesworth et al., 2017). This may be a potential instance of the genetic assimilation first proposed by C.H. Waddington. Low dose radiation is also expected to generate epigenetic (epi- or para-)mutations (Schofield and Kondratowicz, 2017), many of which might become manifest, for example in plants, within one generation (Quadrana and Colot, 2016; Hollick, 2017) and which might be expected to be transmitted through the germ line (van Otterdijk and Michels, 2016). Whether epimutations are likely to be adaptive or maladaptive is still undetermined and may depend on species and circumstances. Because of the potential pleiotropic effects of epimutation an alternative view is that epimutation might be predominantly maladaptive due to existing stabilising selection.

Impacts specifically on developmental processes might also be expected to arise, for example intrauterine environment changes due to environmental insults might confer global effects on fitness and predisposition to disease in adult life through epigenetic mechanisms (Sales et al., 2017). There are insufficient data on dose response *in utero* at low dose to assess if this is a possibility or not, and for which species. There is a paucity of data on internal emitters and placental function in the mammalian species of interest, and little on developmental impact in non-mammalian species. We do not have any informative dose response data on these endpoints. Consequently, it is very difficult to disentangle which mechanisms might dominate at low doses and dose rates.

As an alternative, direct impacts of ionizing radiation on differentiation or tissue function might be important. It is likely that each species will have specific tissues/organs which are more sensitive – e.g. in humans, the haematopoetic cells, the intestinal crypt, the circulatory system and the embryo are especially sensitive. This could have specific impacts, - e.g. diminished brain function, diminished immunity. It will be important to gain an understanding of the "Achilles heel" of each species, particularly focusing on those areas of development, which may affect the ecosystem (e.g. an impact on infection rate).

The shape of the dose response curve is routinely considered in terms of survival, but mutagenesis is also an important outcome. The shape of the dose response curve for mutagenesis is certain to be distinct and also influenced by multiple factors. Firstly, directly induced ionizing radiation damage can lead to small deletions and translocations. The current understanding of mechanisms, might predict that small deletions will arise in a dose independent manner but will arise mainly in heterochromatic DNA (although this has not yet been examined thoroughly). Translocations are known to arise with dose-squared kinetics (upwardly increasing). However, for impacts in an organism, the cell needs to be viable and this also decreases with dose – so the relevant parameter is mutations present in the viable population. Distinct from this are mutations arising indirectly. Currently our understanding of these processes would suggest that indirect mutations (which are likely to be point mutations) will be higher at low doses.

Alternatively, to DNA/mutation dominated ideas in radiation protection other processes related to transcriptomic changes in response to epigenetic modifications could be considered. If we believe in environmental impact on DNA modifications e.g. through DNA methylation with impact on the transcriptome, then we should move forward and identify those changes at the transcriptome level. Since our current knowledge is highly biased by what we are looking for (carcinogenesis with a focus on biological processes such as proliferation, cell death and DNA-repair) we will miss other mechanisms with relevance to the environmental-DNA modifications. Therefore, an agnostic approach with the examination of the whole transcriptome through next generation sequencing would be preferable.

Another consideration is how additional factors, particularly oxidative stress, will affect the shape of the dose response curve for mutagenesis. Finally, the shape of the dose response curve for impacts on epigenetic change or cellular differentiation processes may be different again. We currently have limited data on epigenetic alterations in response to low dose radiation but little on direct involvement in persistent modification of processes of differentiation and cellular function!

From radiation effects on the organism to effects on the whole ecosystem

2.4. Can we identify robust field-based bioassays of effect?

A biomarker is a detectable response (chemical, physical, biological) which can be measured to assess effects and/or exposure. The bioassay is the use of a biological organism to assess effect/exposure. Therefore, in order to develop bioassays for assessing radiation effects, we need to first identify which biomarkers need to be assessed. Certainly, bioassays identifying the effects of ionizing radiation on humans are very well characterised (Pernot et al., 2012) and it is only recently that these approaches are being applied to non-human biota within a radioecological field. There is a huge literature on bioassays for alternative stressors such as heavy metals which could be applied to field-based radioecology. We should be taking advantage of the work that has already been done in these areas. Whereas there are a variety of different measures being conducted in the field (such as single nucleotide polymorphisms, life history, reference), these measures tend to be specific to the study. We should aim to identify approaches that can be applied to a variety of different systems.

The methods that can be applied to field samples such as micronucleus assays, COMET assay etc. are not radiation-specific, so a good approach may be to apply a panel of biomarkers to assess radiation effects. With that in mind, we need to also consider the level at which we are assessing effects (molecular or phenotypic) and consider measuring effects at multiple levels within a study organism (Hinton and Bréchignac, 2005). With the progression of sequencing technologies, there is now a variety of approaches that can be used to investigate even finer-scale changes in the DNA. The difficulty remains in how this might translate to measures of ecosystem level effects. Biodiversity was identified as the key measure of ecosystem-level health in this workshop, however it is important that all confounding factors are considered in these analyses. Particularly in the CEZ, where some of the high radiation environments such as the Red forest provide unique harsh environments incorporating a variety of other stressors.

2.5. What lessons can we learn from Chernobyl and Fukushima regarding extrapolations?

The CEZ and Evacuation designated zone of Fukushima cover, respectively, 2600 and 371 km^2 of land contaminated with different radionuclides following the nuclear accidents in Chernobyl (1986) and Fukushima Dai-ichi (2011) nuclear power plants. An overview of the released total activities and different radionuclides for both sites is given by Steinhauser et al. (2014). Although there are some similarities in the two sites like the evacuation shortly after the accidents and restricted access ever since, both sites are different in many ways (Howard et al., 2017; Steinhauser et al., 2014). As such the total released activity in Chernobyl was about ten-fold that of Fukushima and contained a more complex mixture of radionuclides including less-volatile radionuclides. Since the accidents, extensive research on these sites has been performed and databases have been set up for future use.

A series if comprehensive reviews of the observed (phenotypic) effects in wildlife in CEZ and Fukushima exclusion zone have recently been published e.g. by Hinton et al. (2007), Geras'kin et al. (2008), Lourenco et al. (2016), Steinhauser et al. (2014), Strand et al. (2014); Batlle (2016) and Beresford et al. (2016). In addition, there is considerable "grey" literature and difficult to access Russian literature that still could be investigated and integrated into the databases, further elaborating the knowledge of these sites. It is useful to further work on these two nuclear accident sites as they are unique study areas that will help to understand the long-term impact of ionizing radiation on the environment and gather data that are necessary for modelling. The lessons learned for wildlife exposed for generations to enhanced levels of radiation might help to understand the long-term impact of exposure to humans as well as prepare ourselves for a possible new emergency if this would ever take place.

Care needs, however, to be taken in interpreting data from these highly complex sites. A major issue is the accuracy of dosimetry assessments in the two areas and how to consider the presence of internal emitters, the behaviour of organisms, predator prey relationships and life cycle factors that will lead to different exposures than might be deduced from measurements of ambient dose rates. Both sites contain different habitats, from forest to pastures, and the spatial distribution of the radioactive contamination is highly heterogeneous. The presence of typical Japanese soils like andosols in Fukushima even challenged the existing models based for radionuclide transfer in Ukrainian or European soils (Uematsu et al., 2015, 2016). In addition to radionuclides, other pollutants coming from historical land use in the regions might also be present and need to be considered. It is challenging to find comparable control conditions and little or no information is available of conditions prior to the accidents. Over time both Chernobyl and Fukushima zones have undergone changes induced e.g. by the removal of human presence and occupancy and remediation approaches leading to specific ecological changes that are hard to distinguish from the possible radiological impact (Beresford and Copplestone, 2011; Howard et al., 2017). It is likely that consideration of naturally enhanced sites such as those in Kerala in India and Komi in Russia may generate useful reference data. Taken together both Chernobyl and Fukushima sites are of extreme interest for testing the impact of long-term exposure to radiation but the unique nature of these study areas means that the interpretation of field data from these sites needs careful contextual consideration.

2.6. What are the key knowledge gaps in developing an integrated approach to low dose risk assessment and management?

Radiation and its effects are not particularly well studied except for radiotherapy and in the aftermath of accidents. Much work in the early days of radiation research established that there were effects on human health. Yet there is still a dispute about the effect of dose on different species. Thus, there are some fundamental gaps in knowledge, which impacts on policy and regulations. In terms of low doses of radiation, it is acknowledged that there will be interactions with other pollutants. Consequently, it may be unclear as to what effects should be attributed to radiation in isolation or in combination with other physical, biological or chemical stressors. Clearly, it is not possible to extrapolate the effects of high radiation doses to low levels; the former exerts rapid, acute damages whereas slowly developing chronic changes result with the latter. In terms of accidental releases of radiation, baseline ecological data are largely missing from sites subsequently associated with contamination. Therefore, it is difficult to make meaningful extrapolations of the true effect of the pollution events. More studies are needed to establish baseline knowledge of ecosystems around sites associated with radiation, and in particular to establish baseline biodiversity indices. It is noteworthy that in Belgium, there is a legal requirement for biodiversity surveillance around nuclear power stations. Additionally, work has been carried out, and includes:

- the ecology of bank voles; the radiation event at Chernobyl led to decreased expression of pheomelanin and thus a reduction in red coloration in the skin and hair as a result of the oxidative stress (Boratynski et al., 2014).

False assumptions may be made, with observations contradicting dogma. For example, caesium from Chernobyl was expected to bind to soil, but instead the radionuclides located to some grass species from which absorption occurred (Penrose et al., 2017). Rather than as predicted in adults, children around Chernobyl developed genomic damage (Fucic et al., 2016) and thyroid carcinomas (Drozd et al., 2018) with a link to nitrates in the agricultural area. The EU Habitats Directive discusses the "integrity" of habitats that should be in "good condition". To achieve these laudable aims, it is necessary to adopt interdisciplinary and/or multi-disciplinary approaches to research rather than current more narrowly-focused single subject projects. Radiation research has to be multifaceted encompassing physics, chemistry, biology, radioecology, and epidemiology. There could be an effective role for Citizen Scientists given the existence of new [image recognizing] tools such as involving surveying bats, plants and birds.

Researchers need to be ready for the next nuclear accident.

2.7. Can we identify population and ecosystem level biomarkers or must a "biomarker" always be measured in an organism?

This question defines a key problem, which has held back the field of radiation protection of the ecosystem (as opposed to the organisms within it). The issue is that to make a measurement of a radiation effect and relate it to a dose, both the dose and the endpoint need to be measured accurately. How does one measure a dose to a complex ecosystem and what endpoints indicate harm to an ecosystem? Due to the complexity of this question, the International Commission on Radiological Protection decided to identify reference animals and plants, which represented different habitats and trophic levels as well as different taxonomic groups. Most ecosystem biologists are highly critical of this approach because of the view that it is merely "reference man" expanded to deal with other species, but finding ways to achieve indices of ecosystem health related to dose have proved very difficult. Hinton and Brechignac (2005) suggested identifying a combination of biomarkers assembling various levels of organisation and linking them together as an appropriate approach, but as discussed later this does not capture issues such as system level emergent properties including resilience, redundancy, networking, and signalling. The discussion of this point was wide ranging and included the following ideas:

a. Biodiversity index i.e. the richness of the ecosystem in terms of species diversity could be estimated. There is a large body of information in relation to this due to various biodiversity projects world-wide, and the tools for analysis are well developed (e.g. Escobar et al., 2018, Moresdorf et al., 2018; Jesus et al., 2018; Kissling et al., 2018). The advantage of this endpoint is that it allows a system level endpoint to be measured but can also be designed to focus on nodal points, cornerstone species, which are vital to the condition of that ecosystem. Another advantage is that "citizen science" i.e. natural history records and species databases such as Butterflies and Moths of North America (https://www.butterfliesandmoths.org/) can be used to establish biodiversity richness before a planned release or in a similar habitat should an accidental release occur.
b. Gross measures of ecosystem condition such as transpiration rates, structure of the ecosystem, and the use of drone technology or sophisticated photographic interpretation to detect ecosystem level perturbations (e.g. Li et al., 2014; Zolfagher et al., 2017).
c. Useful methods that could be developed include approaches involving network analysis and the exploration of nodes and connectivity related to resilience. There is a suggestion that stress, including

radiation stress, reduces network complexity (Troncoso-Ponce and Mas, 2012; Nikitaki et al., 2018). Network analysis would permit identification of "collapse points", as was done in fisheries science to predict sustainable levels of fishing.

d. Other suggestions included the use of an "agnostic approach" to biomarkers. This would involve a broad sweep involving many different species in an ecosystem, but looking at the effects in organisms. The new molecular biological toolkits that allow screening for multiple changes and then look for patterns in relation to dose and dose rate of the stressor under investigation lend themselves particularly well to this type of methodology such as the recent screen of microbial populations and communities done by Bálint et al. (2016). Using the "multiple individuals within the ecosystem" approach would also lead to a focus on range and variation in endpoints as a natural consequence of evolutionary changes in wild populations. The current attempts to "explain the causes of uncertainty", (which by definition assume uncertainty is something that can be reduced or eliminated and suggests it is indicative of poor experimental construction or poor data collection) are counterproductive and also misleading. The shape of the "range dose response" rather than the individual dose response should be considered.
e. Multiple stressor appreciation was considered to be a key factor in moving from individual organism to population and ecosystem approaches. While most scientists now agree that low dose radiation is but one stressor among many impacting animal and plant communities, tackling the problem in a meaningful way is very complex. Modelling as discussed later is a promising approach but requires very close interaction of field biologists and mathematicians. Additionally, given the multitude of stressors and species and the millions of possible interactions, it is a so called "wicked problem" (Rittel and Webber, 1973; DeFries and Nagendra, 2017) meaning that the problem is so complex that methods may only become apparent after the answer is known! A more hopeful note might be to suggest that a holistic approach consisting of a combination of the above suggestions or at least an interdisciplinary scientific approach (i.e. translational research from maths, physics, biology) will be needed to tackle this issue.

2.8. How relevant are chemical and pathological stressors in modulating radiation effects?

The discussion centred on fish, which are used as model systems for other organisms. Infectious disease, i.e. caused by bacteria, viruses and parasites, is considered to involve interactions between the host, disease-causing situation, i.e. pathogen, and external stressors (e.g. Austin and Austin, 2016). Stressors in fish pathology may be derived from biological (e.g. toxins from algal blooms), chemical (such as heavy metals, hydrocarbons and pesticides) and physical effects, including temperature increases and radiation. It is unclear for how long or in what quantity stressors are needed to elicit a harmful effect on the host. The direct effect of the stressor may lead to a weakening of the host, including bioaccumulation of the stressor in the host leading to chronic or acute toxicity, immunosuppression, reduced metabolism, physical damage particularly to the gills, or direct interaction with the pathogen. Pollution-related diseases of fish include fin/tail rot, gill disease/hyperplasia, liver damage, neoplasia and ulceration (Austin and Austin, 2016). The sources of information have included surveys and laboratory studies. However, it has been difficult for surveys to definitely link disease with specific pollutants. Moreover, it is unclear if there could be synergism between two or more pollutants, if there is a minimum exposure time, or if there is a threshold time/quantity necessary to elicit harmful effects in the host.

Solar radiation, specifically ultraviolet light, has been linked with photo-enhanced toxicity of hydrocarbons (Alloy et al., 2017). In zebrafish, reduced hatching rate and survival, and developmental abnormalities, i.e. pericardial oedema and deformities, were triggered (Andrade et al., 2017; Barron, 2017). In addition, ulcerative dermal necrosis of salmonids (Poppe, 1989) was mimicked by exposure to ultraviolet light in the presence of phototoxic agents (Bullock and Roberts, 1979); a possibility was that the condition could be akin to sunburn when fish moved from turbid seawater to clear freshwater.

Exposure to gamma irradiation has been directly associated with reduced egg hatching, increased mortalities and deformities, i.e. curvature of the vertebrae and hypertrophy of the yolk-sac in goldfish (Konno et al., 1955). Furthermore, gamma irradiation was linked to altered gene expression (Anbumari and Mohankumar, 2016) and increased susceptibility to fish pathogens (Chilmonczyk and Oui, 1988). Thus, re-activation of dormant *Mycobacterium marinum* infection in zebrafish led to rapid bacterial growth and 88% mortalities within 4-weeks. Radiation caused immunosuppression, namely depletion of granulocytes and monocytes and lymphocyte pools (Parikka et al., 2012). Similarly, exposure to gamma radiation led to the microsporidian parasite *Pseudoloma neurophilia* causing enhanced mortalities in adult zebrafish. The fish displayed enhanced parasite loads and increased myositis, i.e. inflammation of the muscles used for movement (Spagnoli et al., 2016). Within the 30-km Chernobyl nuclear power plant zone a decrease in plants resistance to disease and accelerated development of new phytopathogenic forms and races with enhanced virulence were revealed (Dmitriev et al., 2011).

It remains for further study to elucidate the effect of combinations of stressors, e.g. radiation with chemical, biological or other physical factors. Field trials tend to emphasise single stressors and ignore possible combinations. The problem with laboratory experiments is that they are generally unable to mimic the conditions in the natural environment.

Modelling approaches and novel system level effects relevant in environmental radiation protection

2.9. Do any other mechanisms impact on the spectrum of susceptible species in an ecosystem such as system level emergent mechanisms?

Radiation effects in ecosystems depend on the radiosensitivity of species and the distribution of absorbed doses within an ecosystem. Due to different ways of life and a different ecological niche occupied, doses absorbed by species may vary by several orders of magnitude even when they are all present in the same environment at the same time. Thus, in the first year after an accident doses to biota species may vary significantly, indeed differences of up to 250 times were observed near the Borschevka settlement within the 30-km Chernobyl nuclear power plant zone (Fesenko et al., 2005).

In radiation-impacted ecosystems two groups of effects are identified. In the first period after an accident, when the short-lived radionuclides are the main contributors to the dose, direct effects appear. These types of effects are dependent on the radiosensitivity of the species that an ecosystem comprises. Depending on the dose, direct effects can range from slight inhibition of growth to death or even ecosystem collapse. The ranges of lethal doses for major groups of organisms are extremely wide and cover several orders of magnitude (Whicker and Schultz, 1982). The lethal dose ranges for different groups of organisms only partially overlap. Therefore, while a part of a radiosensitive species may die after irradiation with high doses, vital functions, particularly reproductive efficiency may be suppressed in other species, and radioresistant species may not be harmed and potentially even stimulated. This creates a background for generation of indirect effects in exposed ecosystems.

Irradiation can result in disruption of ecological interactions between components of ecosystems. Such effects may act as a trigger of perturbation and lead to consequences that may differ from those expected from direct effects observed at the organismal level. The typical examples of indirect radiation effects are suppression of radiosensitive species and intensive development of radioresistant ones, disruption of

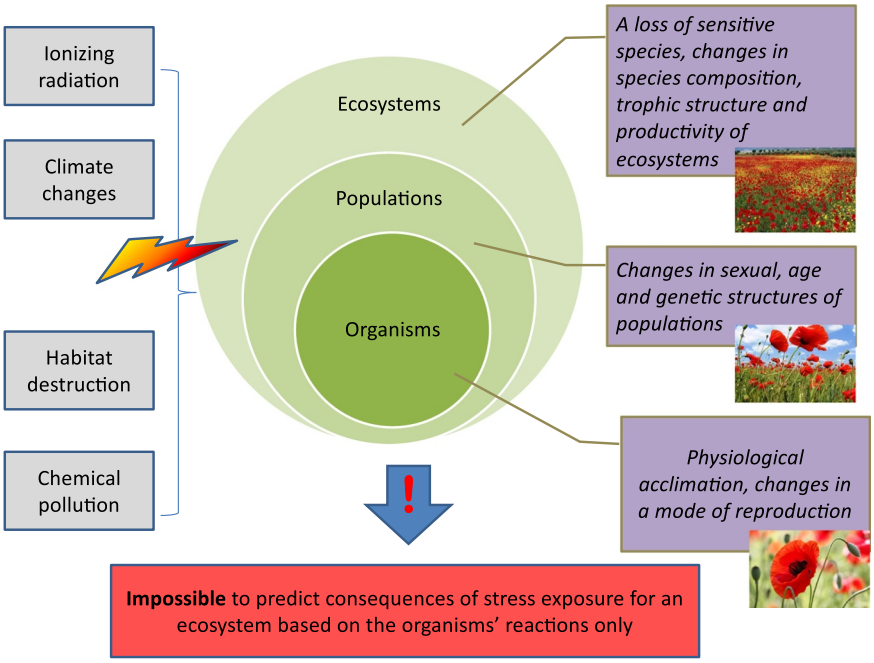

Fig. 1. Effect of radiation-induced alterations in ecosystems.

trophic relations in ecologically connected groups of organisms, an increase in the number of insect pests, and a loss of immunity and emergence of novel infectious diseases.

The type and the magnitude of indirect effects of radionuclides are dependent on ecosystem composition, and many ecological factors other than radiation can be more important. Overall, the disturbance of ecological interrelations may be induced by the following factors:

a. changes in microclimatic and edaphic conditions;
b. disturbance in the synchrony of seasonal phases in development of ecologically connected groups of organisms;
c. an imbalance in relationships between consumers and producers;
d. changes in competition as a result of species differences in radioresistance;
e. a loss of immunity and accelerated development of new pathogenic forms with elevated virulence.

In addition, radiation-induced alterations in ecosystems may open ecological niches for immigration of new species. These indirect effects cannot be deduced solely from the effects on individual organisms (Fig. 1). Therefore, the use of ecological knowledge as supported by an ecosystem approach is essential for understanding responses of populations and ecosystems to radiation.

2.10. If we move to an ecosystem approach how do we deal with dose rate, route of exposure and duration of exposure in the environment, and how can we include concepts of ecosystem resilience, rescue effects and warning/signalling?

In an ecosystem approach, system-level mechanisms of impact (such as are triggered by stressors) will be different from mechanisms of dose-responses at organism level which involve basically molecular and physiological processes (Bréchignac, 2016). These point to the difficulty of extrapolating radiation dose-effect relationships obtained for organisms to predicting how this will affect the population of those organisms (Alonzo et al., 2008; Bréchignac and Doi, 2009; Byrne et al., 2018). Addressing the ecosystem level from a systemic approach may circumvent this difficulty if one aims at demonstrating that populations and ecosystems are actually being protected.

The missing link that an ecosystem approach is capable of fulfilling in comparison to an organism-based approach is to consider species interactions such as predator-prey or primary /secondary consumer interactions (Bradshaw et al., 2014). Species interactions are mandatory not only for an ecosystem's very existence, but also for the survival of its constituent organisms; this is why they largely determine ecosystem structure and functions (such as life support). Several examples of indirect effects of radiation have been published in the recent literature demonstrating that these are widespread in ecosystem responses to stress (Kawagushi et al., 2011; Nascimento and Bradshaw, 2016). They demonstrate that a population can be driven to extinction not by radiation directly, but because its food source species is more radiosensitive. In this context, it is not the dose per se which drives extinction, but a radiosensitivity difference that affects a species interaction inherent to the ecosystem.

Another important system level characteristic of ecosystems, that is entangled with species interactions, is resilience. Resilience is a prime driver of ecosystem response to stress and it can be defined after Holling as "the capacity of a system to absorb disturbance and reorganize while undergoing change so as to still retain essentially the same function, structure, identity, and feedbacks" (Holling, 1973). Resilience is an emergent property of ecosystems that is linked to complex systems theory, and which basically depends upon the species richness (biodiversity), and connectivity of the ecosystem (species interactions). Resilience may be equated with the stability of the system; it is widely accepted that systems with larger biodiversity and highly connected nodes will be more stable. An ecosystem is always an open system and so can never be fully defined. However, some systems may be more fully defined than others. Resilience may be determined more easily in these systems.

The markers of an ecosystem will be determined by the type and nature of the ecosystem and the location (if known) within the ecosystem. In previous examples, aquatic life will have a differential response to river bank life, and mobile life (e.g. voles) will have a difference response to fixed life (plants). It may be that the resilience of a system is a cumulative measurement of the resilience of the components of the system. If one of these components is a "node" (network analysis model), the effect may not be simply cumulative but may be more complicated. Resilience is also often equated with redundancy of

systems. Is that necessarily true in nature where maintenance of exactly the same output might not be critical? So, does resilience in an ecosystem context equate with adaptation?

Prediction of population and ecosystem impairment needs to be simultaneously rooted in knowledge of the dose-response mechanisms happening at an organism level, but also on knowledge about the structure and function of the ecosystem. In particular, indicators such as biodiversity indices, total species biomass, or energy cycling capabilities, which may be affected by stress, could be envisioned. But the goal of these indicators would be to evaluate the susceptibility of the ecosystem to stress as a consequence of its level of resilience. Network analysis, a new field of system level investigations, has the potential to provide a theoretical background along with associated metrics that would allow a rating of these features (Ramos-Jiliberto et al., 2010). Promising investigations are in progress applying this technique to aquatic ponds contaminated with radioactivity to varying degrees (Bradshaw C., personal communication). Network analysis also allows a rating of aspects such as the number of nodes (species) interacting, connectivity and stability, that could be used to identify key critical aspects of the ecosystem in relation to stress that would need particular attention (and that could potentially be used as warning and signalling).

With respect to individual organisms, there are ongoing activities to improve the assessment of dose rates to individuals, for example, through the use of TLDs in the field and capture/recapture experiments so that the time over which the dose is integrated over is known. In addition, for some larger species, there are ongoing studies using TLDs and associated global positioning system units to identify where animals may spend their time in contaminated environments. These studies allow comparisons to be made with the 'standard' approaches to spatially averaging the contamination levels within environmental dose assessments. Initial results have shown that, for some species (Kubota et al., 2015), the (external) dose rate can be estimated reasonably however in other cases, it has become clear that the dose rate may be under- or over-estimated depending upon how individual animals utilise the habitat (for example where food resources may be present) which may be in areas of generally higher or lower contamination levels than the average.

Of course, then there is the issue of using TLDs is that they may not provide a good estimate of the internal dose (usually requires a calibration factor for the position of the TLD relative to the body of the organism in question) and they will not measure the contribution. These types of studies do however start to consider how organisms spend their time in different parts of the ecosystem (e.g. underground, while foraging, in the canopy) so that any estimate of the dose (rate) can take these factors into account. It was therefore noted that, for keystone species and perhaps other societally valued species, we should try to better understand how organisms utilise the environment around them. While this would likely not become the norm for radiological environmental impact assessments, such improved understanding will help to establish the uncertainty within radiological environmental impact assessments and also help to establish the dose response relationships for different biological effects. The question remains, however, as to how representative these estimated dose (rates) are to the population of interest and this will need further consideration however the usefulness of having an 'ecosystem' dose was queried both in terms of being able to compare it to some form of useful dose-response criteria and in terms of communication. It was agreed however that being able to get improved dose estimates to a range of keystone/valued species would be useful.

The Workshop focused on how to improve the context of our understanding of dose rates/doses on individuals and how representative organisms may be (or not) of any ecosystem responses. For example, Alonso-Gonzalez et al. (2016) demonstrated that the radiosensitivity of different ecosystem components to low dose rates can build up to lead to changes at within an ecosystem that are not readily observed at the individual level. It was recommended that improving our understanding of the key linkages between ecosystem components would be beneficial due to increasing recognition of the, generally, subtle changes to timings in key life stages (e.g. timing of flowering and the presence of pollinators) may lead to longer term consequences for ecosystem services (for humans and for the ecosystem itself). Here again, network analysis may enable comparison of ecosystem attributes/components to be undertaken and this may help to identify keystone species or critical ecosystem attributes/components for consideration.

Radioactively contaminated environments, such as affected by a major nuclear accident, exhibit a very uneven spatial distribution of the contamination spanning over a wide range of radionuclides activity concentrations. This feature mechanically leads to a wide range of doses and dose rates to living organisms present in one single population. It follows that the resulting effect on this population cannot easily be rated against dose or dose rate. In addition, when considering the various species forming an ecosystem, accounting for dose or dose rate eliciting potential effects in their populations is further puzzled by their different radio-sensitivities producing different levels of effect for a same dose or dose rate. In this context of wide variations, one can question the sense of averaging dose or dose rate to an ecosystem. It might be more meaningful to consider the total accumulated dose to an ecosystem or its components over a significant number of generations (of some yet to be determined species such as keystone, most radiosensitive) that could be used for comparison purposes.

It must be stressed that addressing responses to low doses of radiation drives a multi-contamination context, with long-term exposure to low levels of other concomitant contaminants. This is indeed the most usual exposure situation for ecosystems where radiation is but one of many stressors at work. Furthermore, the low dose domain eliciting subtle and low effects in organisms, one can question the validity of the low dose-response concept as being the main driver to ecosystem response. Differences in radiosensitivity might be more relevant as they are prone on their own to promote significant system level responses, the actual effect initiated by low dose (at organism level) being simply some kind of a triggering mechanism (Bréchignac, 2017).

2.11. How relevant are adaptive responses and adaptation in populations?

Biological responses to environmental change can involve a variety of mechanisms. These include individual phenotypic plasticity (e.g. developmental plasticity including adaptive acclimation responses). Population, community and ecosystem scale responses may involve evolutionary responses including genetic and epigenetic changes that may be expressed in the organisms carrying them (e.g. as a result of somatic mutations) or in subsequent generations if there is selection, a heritable component to the response, and fitness differences among individual organisms.

Although the term "adaptation" has a diversity of meanings across the biological sciences, most biologists would consider that for evolutionary adaptation to occur, there should be evidence of an increase in fitness across all environments and that changes in phenotype over space should be associated with a change in genotype or epigenotype.

Numerous organisms have shown an ability to survive and reproduce under low-dose radiation arising from natural background radiation or from nuclear accidents. Hence, there is every reason to expect evolution of resistance to radiation even among organisms that have only been irradiated since recent nuclear accidents such as that at Chernobyl in 1986. A number of studies have concluded that there is evidence of adaptation to low dose radiation at Chernobyl. The most persuasive studies have been on bacteria, but studies of birds and trees have been highly suggestive. Studies of bacteria have suggested adaptation to intermediate contamination levels but not high radiation levels suggesting that different mechanisms may be involved at different radiation levels. Studies of birds provide strong evidence that how

organisms deal with oxidative stress may be a key component of any adaptive response (e.g. changes in reduced glutathione allocation are associated with resistance to genetic damage).

It seems likely that all organisms currently living have adapted to some degree to the consequences of ionizing radiation given the near-ubiquity of background radiation on the planet, which in the past has been even higher than current levels. Unfortunately, rigorous tests for evolved adaptive responses often require experimental manipulations of study organisms.

2.12. Evidence suggests that in the field, organisms are more negatively impacted by radiation than in the laboratory; how do we factor this into risk modelling?

Both field and laboratory studies synergistically contribute to understanding and evaluating the basis for environmental assessments. Laboratory studies have been pivotal in establishing mechanistic insights providing understanding of the relationship between the responses and the radioactive contaminants in the environment. Methodologies used in the laboratory are relatively simple and of a more controlled nature. The laboratory outcomes provide insights into pathways of exposure and translation of exposure to biological effects at the genetic, cytogenetic and the physiological levels. Laboratory studies have thus allowed the development of methods of quantification of exposure to radiation (Copplestone et al., 2008). However, the complexities arise with the consideration of the influence of confounding factors, such as food, weather, and the physical and chemical environment, bioavailability of the stressor, also, the reliability of field-dose estimates due to exposure conditions and the species mobility. The field studies are generally based on chronic exposure, whereas laboratory studies usually use acute exposure, which is likely to be another major reason influencing conclusions drawn from field versus laboratory studies. The field evidence although maintaining a high environmental relationship, lacks the power to provide cause-effective relationship between variables. The panoply of factors identified here in this workshop that contributes to the dichotomy between the laboratory and field studies clearly need to be addressed and to be factored in during mathematical modelling. It is unclear if we have sufficient evidence to describe whether organisms are more negatively impacted by radiation in the field compared to the laboratory. However, issues including robust dosimetry and other measurement errors need to be factored in during modelling.

The complex nature of interactions on a population or ecosystem level make it harder to extrapolate the radiation effects observed in the laboratory-based studies. One of the approaches that could be used to extrapolate or understand the radiation damage on ecosystems is mathematical modelling using a systems approach informed and driven by relevant data (Bréchignac et al., 2016). There are examples where a combination of mechanistic modelling with model-guided experiments in a microcosm has proved to be informative in understanding the impact of stressors on this simplified ecosystem (Lamonica et al., 2016). Together with relevant semi-natural, middle-ground experimental and field studies such as microcosms and *meso*cosms, that link laboratory and field studies in ecology (Odum, 1984), modelling can play a pivotal role in radiation risk assessment and prediction. Modelling approaches, such as network analysis and multiscale modelling, can be very useful in studying the key interactions or key interacting nodes within an ecosystem and to factor in the direct and indirect radiation effects. The modelling approaches, that can integrate the effects on an organism level to a population level, can be very useful in generating key hypothesis or relevant questions that could be tested on the field or in the laboratory. Mathematical modelling concepts with multiscale approaches, incorporating changes in multiple levels, spaces and time are already established in several other fields, including cancer and disease modelling and have been found useful in generating testable and valid hypothesis (Powathil et al., 2016; Fernandez-Palomo et al., 2016). The same approach can be used in modelling the complex ecosystem interactions and its role in modulating the radiation effect.

3. Conclusions

It is clear that there is a need to expand the view of ionizing radiation events leading to the effect on individual organisms to the understanding of the interactions of multiple stressors in ecosystems. A multidisciplinary strategy will, therefore, need to be developed. The participants also recognized important knowledge gaps, including having a firm model for the assessment of the relative contributions of classical mutagenic and non-classical non-targeted effects at doses relevant to environmental contamination. Tools need to be developed to tackle the problem of scale (time, space, organisation levels). This means, for example, implement tools that will allow scientists to evaluate risk to populations over generations and within a variety of environments

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